



## Morphological and molecular evidence for a new species of *Russula* (Russulaceae) from southern China

YANG-KUN LI<sup>1</sup>, XIN ZHANG<sup>1,2</sup>, YE YUAN<sup>1</sup>, ZHENG CAO<sup>1,2</sup> & JUN-FENG LIANG<sup>1\*</sup>

<sup>1</sup> Research Institute of Tropical Forestry, Chinese Academy of Forestry, Guangzhou 510520, P. R. China

<sup>2</sup> Southwest Forestry University, Kunming 650224, P. R. China

\*e-mail: [jfliang2000@163.com](mailto:jfliang2000@163.com)

### Abstract

*Russula subbrutilans* sp. nov., a new species of *Russula* is described from southern China. It is unique for having buff pink to light congo-pink pileus, distant ventricose to subventricose lamellae with rare lamellulae, globose to broadly ellipsoid spores with bluntly conical warts forming a partial reticulum, and narrowly clavate to clavate cheilocystidia and pleurocystidia with variable tips. Phylogenetic relationships among the new species and other closely related species in the genus are inferred based on the internal transcribed spacer (ITS) region.

**Keywords:** Basidiomycetes, phylogeny, Russulales & taxonomy

### Introduction

*Russula* Pers. (Russulaceae, Russulales, Basidiomycota), erected by Persoon in 1796, is a widely distributed genus in the world (Persoon 1796; Lebel & Tonkin 2007). Species within *Russula* are well known by the combination of their conspicuous and fleshy fruit bodies, colorful fragile pileus, amyloid warty spores, abundant sphaerocysts in a heteromerous trama that can make the fungi brittle, absence of latex, and the hyphae that lack of clamp connections (Romagnesi 1967, 1985; Singer 1986; Sarnari 1998, 2005). The genus is considered to be of great ecological and economical importance. In forest ecosystems, *Russula* form ectomycorrhizal symbionts with some trees and shrubs of Dipterocarpaceae, Salicaceae, Betulaceae, Pinaceae, Fagaceae, Rosaceae, Fabaceae, Sapotaceae, Nyctaginaceae, Polygonaceae and Tiliaceae (Molina *et al.* 1992; Ying & Zang 1994; Buyck *et al.* 1996). Some species of *Russula* are important edible mushrooms with great commercial values [e.g., *R. rubra* (Fr.) Fr. (1838: 354) and *R. griseocarnosa* Wang *et al.* (2009: 274)] while some in *R. emetica* (Schaeff.) Pers. (1796: 100) group and *R. subnigricans* Hongo (1955: 79) are poisonous (Miller & Buyck 2002; Yang & Piepenbring 2004; Li *et al.* 2010).

The genus *Russula* contains about 750 species and more than 160 of them have been reported from China (Song *et al.* 2007; Kirk *et al.* 2008). However, only 18 species and 3 varieties were originally described from China (Singer 1935; Chiu 1945; Ying 1983; Bi & Li 1986; Ying 1989; Zang & Yuan 1999; Wen & Ying 2001; Song *et al.* 2007; Wang *et al.* 2009; Li *et al.* 2011, 2012, 2013). Because of the lack of systematic study on genus *Russula* in China, many Chinese *Russula* species are misplaced under European or American species names, which probably led to incorrect estimates of the real diversity of *Russula* in China (Li *et al.* 2011, 2012). In this paper, a new species of *Russula* is described from southern China. In order to confirm the phylogenetic uniqueness of *Russula subbrutilans*, sequences of its internal transcribed spacer (ITS) rDNA region were generated and compared with sequences from recent molecular phylogenetic studies of the genus *Russula*.

### Material & methods

#### Sampling

Materials were collected by the authors from China during 2012 and 2014. Notes and photographs were taken on macro-morphological features, and specimens were dried using a dörrex dehydrator at 50°C. Specimens were sealed in

Morphologically, the new species is also closely related to the two species. However, *R. melliolens* differs from *R. subrutilans* by its larger basidiocarp (pileus up to 10 cm), variable tones of stipe, bigger spores ( $8.5\text{--}11.2 \times 8\text{--}9.5 \mu\text{m}$ ), crowded and white lamellae discolouring yellowish or ochraceous with age, and cylindrical to fusiform pleurocystidia with long tapering apices (Romagnesi 1967), while *R. umerensis* by its pallid grayish violet pileus, crowded lamellae, conspicuous plage of spores, and fusiform pleurocystidia with the bluntly acuminate (McNabb 1973).

Another species of sect. *melliolentinae*, *Russula viscida* Kudřna (1928: 56), which described from Czech, is worth noting here. However, the species is not clustered with *R. melliolen* and *R. subrutilans* from phylogenetic analyses. Moreover, *R. viscida* has a bigger basidiocarp (pileus  $\geq 5.5$  cm), a red purple tone pileus, crowded lamellae, spores with almost complete reticulum and fusiform cystidia, which make it easily to be distinguished from *R. subrutilans* (Romagnesi 1967).

Furthermore, several species easily to be confused with *R. subrutilans* in field are also compared. *Russula arpalices* Sarnari (1994: 12), described from Italy, is very similar to *R. subrutilans* in field, but the former species can be recognized by its mature pileus often discoloured brownish ochre, slender stipe readily discolored ochraceous to ochre-brown, larger and narrower spores ( $7.5\text{--}9.2 \times 5.9\text{--}6.8 \mu\text{m}$ ) with warts forming a complete reticulum and distinctive Pelargonium-like odor (Sarnari 2005, Aron & Kibby 2013). *R. zonatula* Ebbesen & Jul. Schäff (1952: 260) is distinguished by its obviously discolored pileus and center often with deep red or purple tone, spore ornamentation isolated, larger pleurocystidia (up to  $90 \mu\text{m}$ ), and is associated with beech forest (Romagnesi 1967). *Russula minutula* Velen. (1920: 133) has smaller basidiocarp ( $\leq 3.0$  cm), hollow stipe, smaller spores ( $5.7\text{--}7.7 \times 5\text{--}6.7 \mu\text{m}$ ) and basidia ( $\leq 35 \mu\text{m}$ ; Romagnesi 1967), while *Russula minutula* var. *minor* Bi (1986: 195) has a smaller basidiomata ( $\leq 2.0$  cm), hollow stipe, broadly ellipsoid spores with isolated warts or rare ridges, white spore print, smaller cystidia (Bi & Li 1986, Li 2013). *Russula luteotacta* Rea (1922: 469) is distinguished by its slender stipe (up to 7.0 cm), white spores print and spores with warts isolated or joined in rows to form ridge but not forming a partial reticulum, fusoid pleurocystidia with obtuse tip, narrower basidia ( $\leq 11 \mu\text{m}$ ) and larger pileocystidia ( $\geq 70 \mu\text{m}$ ; Rea 1922, Romagnesi 1967) while *Russula betularum* Hora (1960: 456) by its separable cuticle, hollow stipe, hymenial cystidia with the tips various tapering and spore warts isolated or weak lines (Hora 1960, Tschen & Tschen 2005).

## Acknowledgements

We are grateful to Dr. Zhijiao Song for improving the manuscript. Our most sincere thanks also give Shichao Liu, Fuyan Liu, Ye Tian and Zhenbo Gao for the language polishing. This study was supported by the National Nonprofit Institute Research Grant of CAF (CAFYBB2014MA003) and the National Natural Science Foundation of China (No. 31070014).

## References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.  
[http://dx.doi.org/10.1016/S0022-2836\(05\)80360-2](http://dx.doi.org/10.1016/S0022-2836(05)80360-2)
- Aron, C. & Kibby, G. (2013) *Russula arpalices* new to Britain. *Field Mycology* 14(3): 93–95.  
<http://dx.doi.org/10.1016/j.fldmyc.2013.06.009>
- Bi, Z.S. & Li, T.H. (1986) A preliminary note on *Russula* species from Guangdong, with a new species and a new variety. *Guihaia* 6(3): 193–199.
- Buyck, B., Thoen, D. & Watling, R. (1996) Ectomycorrhizal fungi of the Guinea–Congo region. Proceedings of the Royal Society of Edinburgh. Section B. *Biological Sciences* 104: 313–333.  
<http://dx.doi.org/10.1017/S0269727000006175>
- Chiu, W.F. (1945) The Russulaceae of Yunnan. *Lloydia* 8: 31–59.
- Durall, D.M., Gamiet, S., Simard, S.W., Kudřna, L. & Sakakibara, S.M. (2006) Effects of clearcut logging and tree species composition on the diversity and community composition of epigeous fruit bodies formed by ectomycorrhizal fungi. *Botany* 84(6), 966–980.  
<http://dx.doi.org/10.1139/b06-045>
- Edgar, R.C. (2004) MUSCLE multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–1797.

<http://dx.doi.org/10.1093/nar/gkh340>

- Fries, E.M. (1836) *Anteckningar öfver de i Sverige växande ätliga svampar*. Palmblad, Sebell, Uppsala, Sweden, 50 pp.
- Fries, E.M. (1838) *Epicrasis systematis mycologici, seu synopsis Hymenomycetum*. Typographia Academica, Uppsala, Sweden, 354 pp.  
<http://dx.doi.org/10.1080/00222934009512452>
- Fries, E.M. (1863) *Monographia hymenomycetum Sueciae 2*. CA Leffler, Uppsala, 194 pp.
- Hongo, T. (1955) Notes on Japanese larger Fungi (6). *Journal of Japanese Botany* 30 (3): 73–79.
- Guo, J., Karunarathna, S.C., Mortimer, P.E., Xu, J. & Hyde, K.D. (2014) Phylogenetic Diversity of *Russula* from Xiaozhongdian, Yunnan, China, Inferred from Internal Transcribed Spacer Sequence Data. *Chiang Mai Journal of Science* 41(4), 811–821.
- Hora, F.B. (1960) New check list of British agarics and boleti: Part IV. Validations, new species and critical notes. *Transactions of the British Mycological Society* 43 (2): 440–459.  
[http://dx.doi.org/10.1016/S0007-1536\(60\)80067-8](http://dx.doi.org/10.1016/S0007-1536(60)80067-8)
- Huelskenbeck, J.P. & Ronquist, F. (2005) Bayesian analysis of molecular evolution using MrBayes. In: Nielsen, R. (Ed.) *Statistical methods in molecular evolution*. Springer New York, pp. 183–226.  
[http://dx.doi.org/10.1007/0-387-27733-1\\_7](http://dx.doi.org/10.1007/0-387-27733-1_7)
- Huson, D.H., Richter, D.C., Rausch, C., DeZulian, T., Franz, M. & Rupp, R. (2007) Dendroscope: An interactive viewer for large phylogenetic trees. *BMC bioinformatics* 8 (1): 460.  
<http://dx.doi.org/10.1186/1471-2105-8-460>
- Jones, M.D., Phillips, L.A., Treu, R., Ward, V. & Berch, S.M. (2012) Functional responses of ectomycorrhizal fungal communities to long-term fertilization of lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) stands in central British Columbia. *Applied Soil Ecology* 60: 29–40.  
<http://dx.doi.org/10.1016/j.apsoil.2012.01.010>
- Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008) *Dictionary of the fungi. 10th Edition*. CABI International, Oxon, UK, pp. 608–609.
- Klavina, D., Gaitnieks, T. & Menkis, A. (2013) Survival, growth and ectomycorrhizal community development of container-and bare-root grown *Pinus sylvestris* and *Picea abies* seedlings outplanted on a forest clear-cut. *Baltic Forestry* 19 (1): 39–49.
- Lebel, T. & Tonkin, J.E. (2007) Australasian species of *Macowanites* are sequestrate species of *Russula* (Russulaceae, Basidiomycota). *Australian Systematic Botany* 20 (4): 355–381.  
<http://dx.doi.org/10.1071/SB07007>
- Li, G.J. (2013) *Taxonomy of Russula from China*. PhD dissertation, Institute of Microbiology, University of Chinese Academy of Sciences, Beijing.
- Li, G.J., Li, S.F., Liu, X.Z. & Wen, H.A. (2012) *Russula jilinensis* sp. nov. (Russulaceae) from northeast China. *Mycotaxon* 120: 49–58.  
<http://dx.doi.org/10.5248/120.49>
- Li, G.J., Li, S.F. & Wen, H.A. (2011) *Russula zhejiangensis* sp. nov. from East China. *Cryptogamie Mycologie* 32 (2): 127–133.  
<http://dx.doi.org/10.7872/crym.v32.iss2.2011.127>
- Li, G.J., Zhao, D., Li, S.F., Yang, H.J. & Liu, X.Z. (2013) *Russula changbaiensis* sp. nov. from northeast China. *Mycotaxon* 124 (1): 269–278.  
<http://dx.doi.org/10.5248/124.269>
- Li, G.J., Zhao, Q., Zhao, D., Yue, S.F., Li, S.F., Wen, H.A. & Liu, X.Z. (2013) *Russula atroaeruginea* and *R. sichuanensis* spp. nov. from southwest China. *Mycotaxon* 124 (1): 173–188.  
<http://dx.doi.org/10.5248/124.173>
- Li, M.C., Liang, J.F., Li, Y.C., Feng, B., Yang, Z.L., James, T.Y. & Xu, J.P. (2010) Genetic diversity of Dahongjun, the commercially important “Big Red Mushroom” from southern China. *PLoS one* 5 (5): e10684.  
<http://dx.doi.org/10.1371/journal.pone.0010684>
- McNabb, R.F.R. (1973) Russulaceae of New Zealand 2. *Russula* Pers. ex SF Gray. *New Zealand Journal of Botany* 11 (4): 673–730.  
<http://dx.doi.org/10.1080/0028825X.1973.10430308>
- Miller, S.L. & Buyck, B. (2002) Molecular phylogeny of the genus *Russula* in Europe with a comparison of modern infrageneric classifications. *Mycological Research* 106 (3): 259–276.  
<http://dx.doi.org/10.1017/S0953756202005610>
- Molina, R., Massicotte, H. & Trappe, J.M. (1992) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. *Mycorrhizal functioning: an integrative plant-fungal process* 375: e423.
- Osmundson, T.W., Robert, V.A., Schoch, C.L., Baker, L.J., Smith, A., Robich, G., Luca, M. & Garbelotto, M.M. (2013) Filling gaps in biodiversity knowledge for macrofungi: contributions and assessment of an herbarium collection DNA barcode sequencing project. *PLoS one* 8 (4): e62419.  
<http://dx.doi.org/10.1371/journal.pone.0062419>

- Palmer, J.M., Lindner, D.L. & Volk, T.J. (2008) Ectomycorrhizal characterization of an American chestnut (*Castanea dentata*)-dominated community in Western Wisconsin. *Mycorrhiza* 19 (1): 27–36.  
<http://dx.doi.org/10.1007/s00572-008-0200-7>
- Persoon, C.H. (1796) *Seu, Descriptiones tam novorum quam notabilium fungorum*. Wolf, Lipsiae. Observations Mycologicae, 100 pp.
- Posada, D. & Buckley, T.R. (2004) Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793–808.  
<http://dx.doi.org/10.1080/10635150490522304>
- Quélet, L. (1898) Quelques espèces critiques ou nouvelles pour la Flore mycologique de France. *Comptes Rendus de l'Association Française pour l'Avancement des Sciences* 26 (2): 446–452.
- Rea, C. (1922) *British Basidiomycetae: a handbook to the larger British fungi*. Cambridge University Press, Cambridge, 469 pp.
- Ridgway, R. (1912) *Color standards and color nomenclature*. Robert Ridgway, Washington.
- Romagnesi, H. (1967) *Les Russules d'Europe et d'Afrique du Nord*. Bordas, Paris.
- Romagnesi, H. (1985) *Les Russules d'Europe et d'Afrique du Nord*. Reprint with supplement. J. Cramer, Lehre.
- Sarnari, M. (1994) Russula nuove o interessanti dell'Italia centrale e mediterranea-XXIV contributo. *Bollettino dell'Associazione Micologica ed Ecologica Romana* 30–31: 8–13
- Sarnari, M. (1998) *Monografia illustrate de genere Russula in Europa*. Tomo Primo. AMB, Centro Studi Micologici, Trento.
- Sarnari, M. (2005) *Monografia illustrate de genere Russula in Europa*. Tomo Secondo. AMB, Centro Studi Micologici, Trento.
- Schaeffer, J. (1952) *Russula-Monographie*. J. Cramer, 260 pp.
- Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A. & Chen W. (2012) The internal transcribed spacer as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences* 109 (16): 6241–6246.  
<http://dx.doi.org/10.1073/pnas.1117018109>
- Singer, R. (1935) Supplemente zu meiner Monographie der Gattung *Russula*. *Annales Mycologici* 33: 297–352.
- Singer, R. (1986) *The Agaricales in modern taxonomy*. 4th ed. Koeltz Scientific Books, Koenigstein, 981 pp.
- Song, B., Li, T.H., Wu, X.L., Li, J.J., Shen, Y.H. & Lin, Q.Y. (2007) Known species of *Russula* from China and their distribution. *Journal of Fungal Research* 5 (1): 20–42.
- Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology* 57: 758–771.  
<http://dx.doi.org/10.1080/10635150802429642>
- Teasdale, S. E., Beulke, A. K., Guy, P. L., & Orlovich, D. A. (2013) Environmental barcoding of the ectomycorrhizal fungal genus *Cortinarius*. *Fungal Diversity* 58 (1): 299–310.  
<http://dx.doi.org/10.1007/s13225-012-0218-1>
- Tschen, E.F.T. & Tschen, J.S.M. (2005) Three species of *Russula* new to Taiwan. *Fungal Science* 20 (1&2): 47–52.
- Velenovský, J. (1920) *Ceské Houby I*. České Botanické Společnosti, Prague, 133 pp.
- Vellinga, E.C. & Noordeloos, M.E. (2001) Glossary. In: Noordeloos, M.E., Kuyper, T.W. & Vellinga, E.C. (Eds.) *Flora agaricina neerlandica*. 5. A.A. Balkema Publishers, Rotterdam, pp. 6–11.
- Wang, X.H., Yang, Z.L., Li, Y.C., Knudsen, H. & Liu, P.G. (2009) *Russula griseocarnosa* sp. nov. (Russulaceae, Russulales), a commercially important edible mushroom in tropical China: mycorrhiza, phylogenetic position, and taxonomy. *Nova Hedwigia* 88: 269–282.  
<http://dx.doi.org/10.1127/0029-5035/2009/0088-0269>
- Wen, H.A. & Ying, J.Z. (2001) Study on the genus *Russula* Pers from China II. Two new taxa from Yunnan and Guizhou. *Mycosystema* 20 (2): 153–155.
- White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*. Acad Press, San Diego, pp. 315–322.
- Yang, Z.L. & Piepenbring, M. (2004) *Wild edible fungi in the Yunnan Province, southwestern China*. Frontiers in Basidiomycete mycology, pp. 419–426.
- Ying, J.Z. (1983) A study on *Russula viridi-rubrolimbata* sp. nov. and its related species of subsection virescentinas. *Mycosystema* 2 (1): 34–37.
- Ying, J.Z. (1989) Study on the genus *Russula* Pers from China I. New taxa of *Russula* from China. *Mycosystema* 8 (9): 205–209.
- Ying, J.Z. & Zang, M. (1994) *Economic macrofungi of southwestern China*. Science Press, Beijing, 399 pp.
- Zang, M. & Yuan, M.S. (1999) Contribution to the knowledge of new basidiomycetous taxa from China. *Acta Botanica Yunnanica* 21 (1): 37–42.
- Zhou, L.L. & Liang, J.F. (2011) An improved protocol for extraction of DNA from macrofungi. *Guangdong Forest Science Technology* 27: 13–16.